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FINAL TECHNICAL REPORT

"Functional significance of drug-induced changes in brain monoamine levels"

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Abstract.

The mechanism of monoaminergic (i.e. adrenergic, noradrenergic, dopaminergic, and serotonergic) transmission in general is discussed on the basis of observations on the effects of various agents (chiefly precursors and precursor analogs, inhibitors of enzymes responsible for synthesis and degradation of monoamines, and drugs interfering with storage and release). The storage granules (or vesicles) of the monoaminergic nerve terminals, in which the transmitter can be visualized under the fluorescence microscope by means of a new histochemical technique, appear to have a dual function, i.e. a) to serve as a store of transmitter, and b) to make newly synthesized transmitter available for release by membrane depolarization. The former function does not seem to be essential, since the store can be depleted without any disturbance of transmission. The latter function seems to be essential: block of the uptake mechanism of the granules by reserpine results in block of transmission.

In the past decade numerous attempts have been made to correlate changes in monoamine levels in brain, induced e.g. by drugs, with changes in behavior and other brain functions. The results have been variable. For example, a fall in the level of one of the monoamines may coincide with sedation, excitation or no change in behavior. It is thus evident that no strict correlation exists. It would of course be erroneous to conclude from this that the monoamines of brain are of no functional importance. On the contrary, we have good reasons for assuming that the catecholamines as well as 5-hydroxytryptamine (5 HT) serve as transmitters in brain (Falck 1962, Carlsson, A., Falck, B., and Hillarp, N.-Å., 1962). There are also good reasons for a poor correlation. The major part of the monoamines in brain occur in an inactive store, and the level of this store may vary independently of the level of free and active monoamines in the extracellular space near the receptor sites of the effector neurons. The situation is further complicated by the fact that the monoamine stores may consist of at least two different fractions, as demonstrated first by Hillarp (1960) on adrenal medullary granules. In addition, as Dr. Falck will show, part of the stored transmitter may be located at an appreciable distance from the effector cells.

I should like to discuss first the functional significance of the monoamine stores: how much can they be reduced without impairment of function?

Comparison between Reserpine and α-Methyl Metatyrosine (α-MMT).

As is well known, there are two types of drugs which cause particularly marked depletion of tissue monoamines, namely, the Rauwolfia alkaloids and benzoquinolizines on one hand, and the DOPA analogues α-methyl DOPA and α-methyl metatyrosine (α-MMT) on the other. While the former group acts on the catecholamines as well as 5 HT, the latter acts fairly selectively on noradrenaline (Hess et al., 1961, Porter et al., 1961). A common feature of reserpine and these DOPA analogues is that both cause a marked depletion of noradrenaline in central as well as peripheral noradrenergic neurons. From the technical point of view the peripheral noradrenergic neurons have the great advantage that their function can be easily studied. As is well known, the peripheral noradrenergic neurons cease to function under the influence of reserpine, when given in doses which cause depletion of the adrenergic transmitter. We know of no data in the literature dealing with the function of the adrenergic nerves after severe depletion of the transmitter by α-methyl DOPA or α-MMT. Stone et al., (1962), have done some studies along this line in dogs, but with the doses used the noradrenaline levels in tissues dropped only by about 50 per cent. Experiments with α-MMT in doses causing severe depletion have therefore been carried out in our laboratory (Andén and Magnusson, unpublished experiments). It was found that in order to obtain almost complete depletion of noradrenaline α-MMT had to be given in large repeated doses (400 mg/kg daily for 2 or 3 days). In fact, maximum effect was obtained if, in addition, a small dose of metaraminol was given intravenously about 4 hours before the experiment (0,2 mg/kg). (The action of α-MMT is largely mediated through metaraminol, Carlsson and Lindqvist, 1962 a and b.) Under such conditions some 97 per cent depletion of noradrenaline was obtained in brain, heart, and spleen of rats and cats and in the iris and nictitating membrane of cats, using the extremely sensitive method of Häggendal, (1963 a). In no instance has it been

possible to block the noradrenergic transmission mechanism, irrespective of whether α-MMT has been given in single or repeated doses or whether it has been given alone or in combination with metaraminol. The function of the noradrenergic nerves has been studied in several ways, mostly after unilateral cervical sympathectomy. Lack of ptosis, miosis, and relaxation of the nictitating membrane on the intact side, while at the same time these symptoms have been present on the side of cervical sympathectomy has been taken as indication of persistent sympathetic activity. Protrusion of the eyeball, dilatation of the pupils, and contraction of the nictitating membrane following electrical stimulation of the cervical sympathetic has been taken as evidence of an intact noradrenergic transmission mechanism. Furthermore, the rise in blood pressure following electrical stimulation of the splanchnic nerves, carotid occlusion, injection of tyramine or carbachol after atropine in adrenalectomized or demedullated animals has been used to investigate the noradrenergic transmission mechanism. The experiments have been performed in cats as well as rats. The experiments showed conclusively that α-MMT was unable to impair the noradrenergic transmission mechanism, even in doses which caused the virtually complete depletion of the stores of noradrenergic transmitter. There thus appears to be a fundamental difference in the mode of action of reserpine and α-MMT. As to the mode of action of α-MMT, the following 3 alternatives may be considered.

1) For the functions mentioned the sympathetic system may not be entirely dependent on the noradrenergic transmitter but may work partly through other transmitters as well. This alternative might be seriously considered if α-MMT had caused some reduction of sympathetic activity. However, the sympathetic system seemed to function quite normally. This alternative therefore seems unlikely.

2) The decarboxylation products of α-MMT, i.e. α-methyl metatyramine and α-methyl β-hydroxy metatyramine or metaraminol may be stored in the nerve endings and take over the functions of the adrenergic transmitter.

At the First International Pharmacological Meeting in Stockholm, August 1961, we reported that the prolonged depletion of noradrenaline caused by α-methyl DOPA and α-MMT was mediatdd by their respective decarboxylation products (Carlsson, 1962; cf. Carlsson and Lindqvist, 1962 a and b, see Fig. 1). This was not generally accepted at first, but now there seems to be general agreement that this is so. It has been admitted also by Costa et al., as is evident e.g. from the printed, considerably revised version of their presentation at the same Meeting (1962 a). However, one point remains to be discussed, namely, the mechanisms by which these amines cause depletion of noradrenaline. In our first report we presented evidence to support the view that displacement had taken place: the amounts of α-methylated amines (at late intervals as 24 hours or more the β-hydroxy derivatives dominated) found were of the same order as the missing noradrenaline. This has been disputed by Costa et al., (1962 a), as well as by Udenfriend and Zaltsman-Nirenberg, (1962). According to these investigators, the amounts of decarboxylation products formed from α-MMT are small and disappear rapidly, usually within 24 hours, while the depletion of noradrenaline persists for several days. We have repeated several of the experiments reported by these investigators (see also Gessa et al., 1962 a and b, Brodie et al., 1962 b, Costa et al., 1962 b), and in all

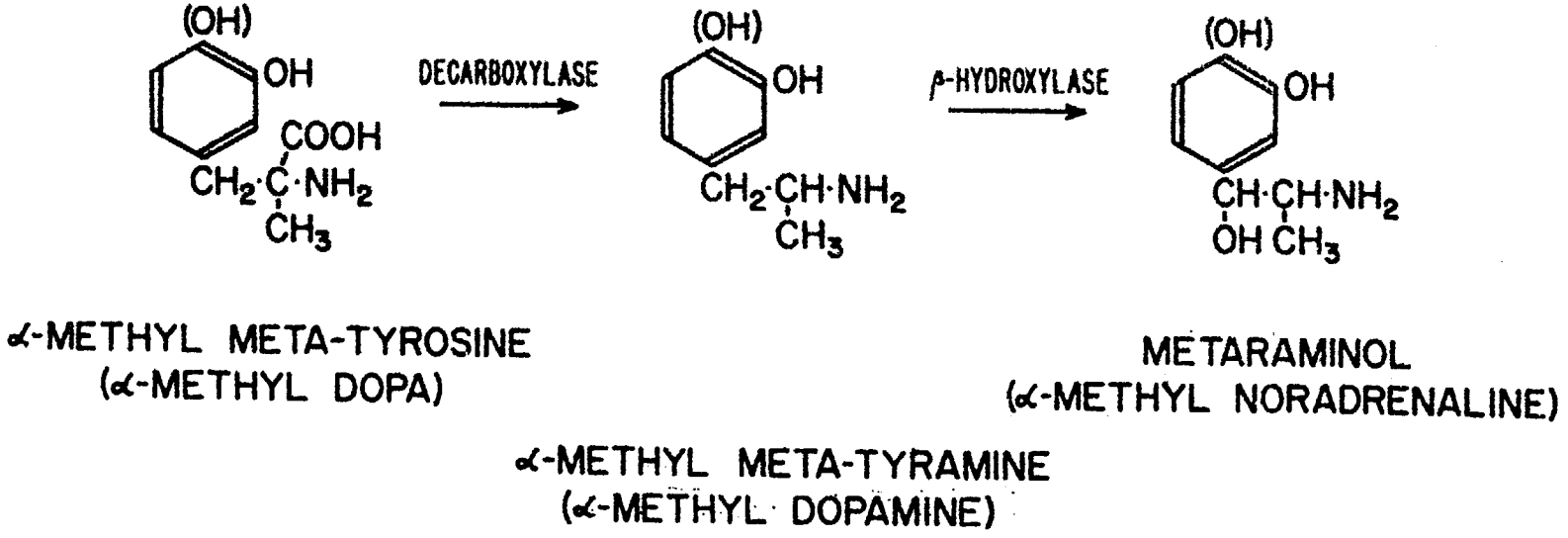


Figure 1. α-Methyl DOPA, α-Methyl Metatyrosine and Their Decarboxylation Products.

instances we find much larger amounts of decarboxylation products than they have found. For example, in the brains of rabbits given α-MMT in a dose of 100 mg/kg intravenously, metaraminol was found in amounts corresponding roughly to the missing noradrenaline as late as 7 days after the injection (Fig. 2). According to our results displacement plays an important role in the depletion of noradrenaline by α-MMT (and α-methyl DOPA). Other factors, e.g. inhibition of synthesis, seem to contribute, however. This will be discussed later.

The possibility that α-methyl metatyramine and metaraminol act as substitutes for the noradrenergic transmitter, must therefore be considered. Certain facts argue against this mechanism as being the only or even the chief factor, however, Metarminol is a much weaker sympathomimetic agent than noradrenaline. This might be compensated by increased release, but this probably does not occur, since metaraminol actually appears to remain in the stores much longer than noradrenaline. Furthermore, it has been found that D-adrenaline is an efficient displacer of noradrenaline (Andén, unpublished experiments). Even after the almost complete depletion of noradrenaline by D-adrenaline the noradrenergic nerves appear to function normally, as judged by the criteria just mentioned (Andén and Magnusson, unpublished experiments). The physiological activity of D-adrenaline is much lower than that of L-noradrenaline. It might be argued that this is compensated by a more rapid liberation, but this is unlikely as the disappearance of L- and D-adrenaline occur at about equal rates.

3) A third explanation as to why noradrenergic transmission appears to be intact even after the virtually complete depletion of the transmitter by α-MMT (and α-methyl DOPA and D-adrenaline) is that the major part of the store of noradrenergic transmitter is not essential for the transmitter function. If this is the correct alternative, it remains to explain why reserpine blocks the noradrenergic transmission.

It has long been known that the sedative action of reserpine is not strictly correlated with the monoamine levels in brain. This is particularly true of the recovery stage, where functional recovery is reached while the monoamines are still very low. In fact, it has proved possible to keep the monoamine levels in rabbit brain very low by small daily doses of reserpine (0.2 mg/kg subcutaneously) with but slight functional impairment (Häggendal and Lindqvist, 1962). These animals are somewhat sedated for some 6 hours after each injection, but after 24 hours some very slight miosis and photophobia are the only conspicuous signs of reserpine effect, in spite of the fact that the monoamine levels in brain even at this interval are very low (about 10 per cent of normal). It is, however, of interest to note that in these chronically treated animals the monoamine levels are significantly lower at the time of sedation (after 4 hours) than after 24 hours. After a single dose rather the reverse is true. This phenomenon deserves further investigation with chemical analyses and histochemical examination in parallell.

Although a direct action of reserpine on platelet 5 HT was demonstrated many years ago by Brodie and his coworkers - I had the privilege to be one of them (Carlsson et al., 1957), - it has only recently become possible to demonstrate a direct action of the drug on specific storage granules. This has been unfortunate because the suspicion that such an effect does not exist, has caused confu-

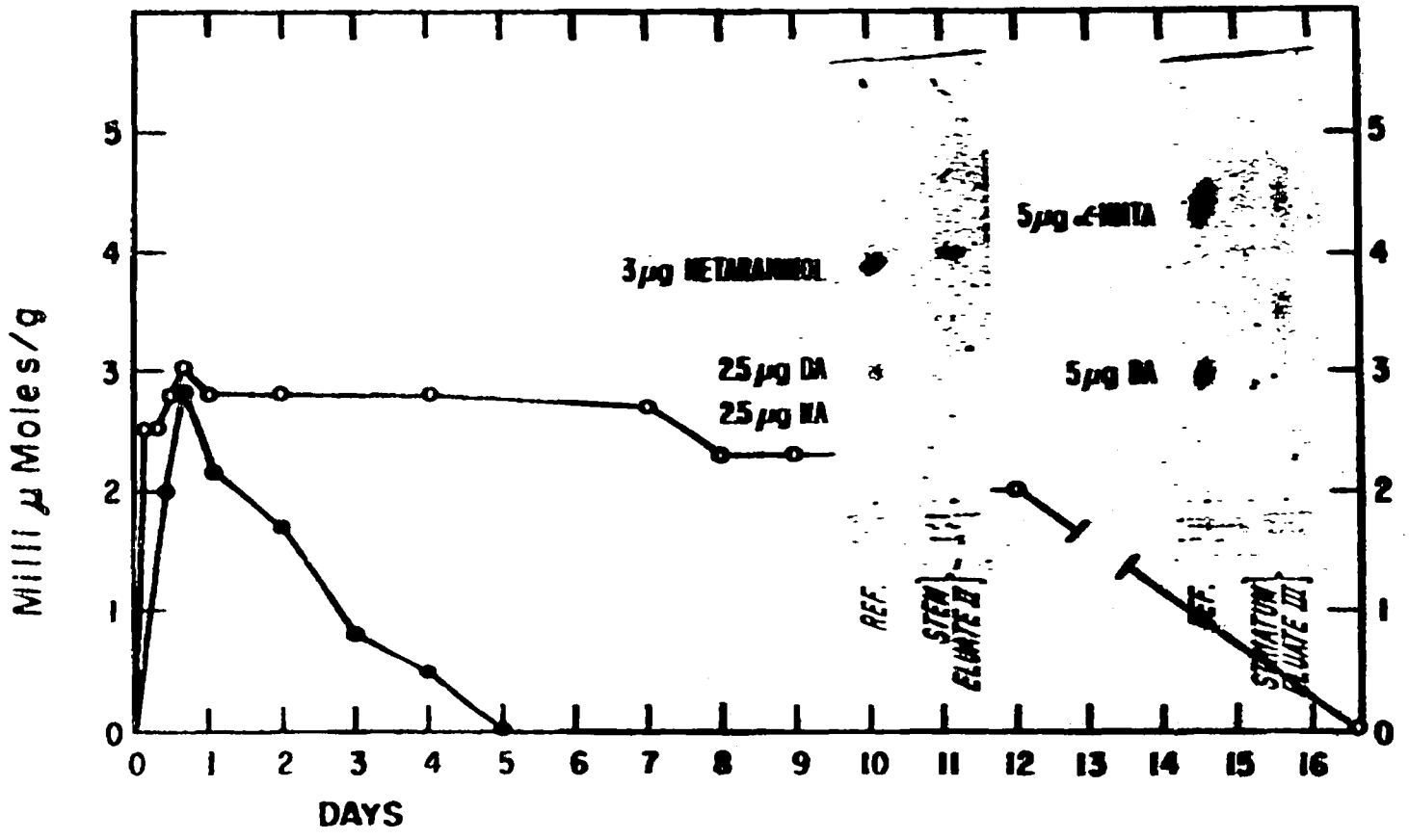


Figure 2. Insert. Paper Chromatograms of the Present Laboratory Demonstrating Metaraminol (left) and α-Methyl Metatyramine (right) in Brain Stem and Striatum, respectively, of Rabbits Injected with α-Methyl Metatyrosine (100 mg/kg i.v.) 7 Days Previously (for techniques, see Carlsson and Lindqvist, 1962 a). - Graph from Gessa et al. (1962 b) Demonstrating Absence of These Decarboxylation Products in Brain Stem of Rabbits Under the Same Experimental Conditions. - The amount of metaraminal found on the chromatogram is of the same order as the missing noradrenaline.

sion. As a result of independent work of Kirshner (1962), and Hillarp and coworkers (Carlsson, Hillarp and Waldeck, 1962, and unpublished experiments) we now know that if adrenal medullary storage granules are incubated together with labelled noradrenaline (adrenaline, dopamine, or 5 HT) in low concentration together with some ATP and Mg ions, the granules incorporate the amino at a fairly high rate. The ATP does not seem to be incorporated in stoichiometric amounts, so it appears that the incorporation occurs primarily in the labile ATP-free fraction discovered by Hillarp (1960). This incorporation is blocked by reserpine, when added in low concentration to the suspension medium.

If adrenal medullary granules are examined in this way at different intervals following injection of a single dose of reserpine (5 mg/kg intravenously) to rabbits, incorporation of labelled amine is blocked 12 to 24 hours after the injection. After about 48 hours the incorporation is restored to normal in spite of the fact that the adrenal medulla is still completely depleted of catecholamines (unpublished experiments, Fig. 3). In other words, restoration of the storage function precedes that of amine levels. It appears that storage function rather than amine levels is correlated with sedation and other pharmacological effects of reserpine. This suggests that amine must be taken up by the granules before it can be released by nerve activity.

When all the data are considered together, the following hypothetical picture emerges (Fig. 4). Normally the precursor amino acid enters the cell and is decarboxylated. Alternatively the amine enters the cell from the extracellular space. The amine then enters the storage granule, where it is incorporated into the labile fraction. The presence of ATP and Mg ions is required for this incorporation. Part of the labile fraction may then be incorporated or converted into the stable fraction or, alternatively, released into the extracellular space by an influence of the action potential on the storage granule. There may also be some back-leakage to the cytoplasmic sap and monoamine oxidase (MAO). Reserpine blocks the incorporation into the labile fraction. Synthesis is going on but the amine formed is destroyed in the cell by the MAO of the mitochondria. Depletion of the labile fraction is delayed by replenishment from the stable fraction. Consequently, nerve transmission is still possible for some time. However, finally the labile fraction disappears and nerve transmission ceases. During recovery newly formed amine is incorporated into the labile fraction, which is immediately utilized, i.e. released by nerve activity. In other words, transmission is restored before the amines accumulate.

The decarboxylation products of α-methyl DOPA and α-MMT, as well as D-adrenaline conceivably enter the labile fraction first, and later the stable fraction. However, noradrenaline continues to be synthesized, and its incorporation into the labile fraction is not blocked. Transmission is therefore unimpaired.

In any case it is evident that the use of drugs as tools for clarifying the functions of the monoamines may easily lead to erroneous conclusions. For example, it cannot be expected that a drug like α-MMT which causes depletion of monoamine stores without interfering with the transmission mechanism, should affect behavior in the same manner as reserpine, which is capable of blocking transmission.

In brain the situation is rendered even more difficult through the fact that, for example, noradrenaline-containing neurons occur in a variety of functionally more or less independent systems, which show marked differences in sensitivity to drugs such as reserpine. This has been revealed by histochemical work (Falck, This Symposium).

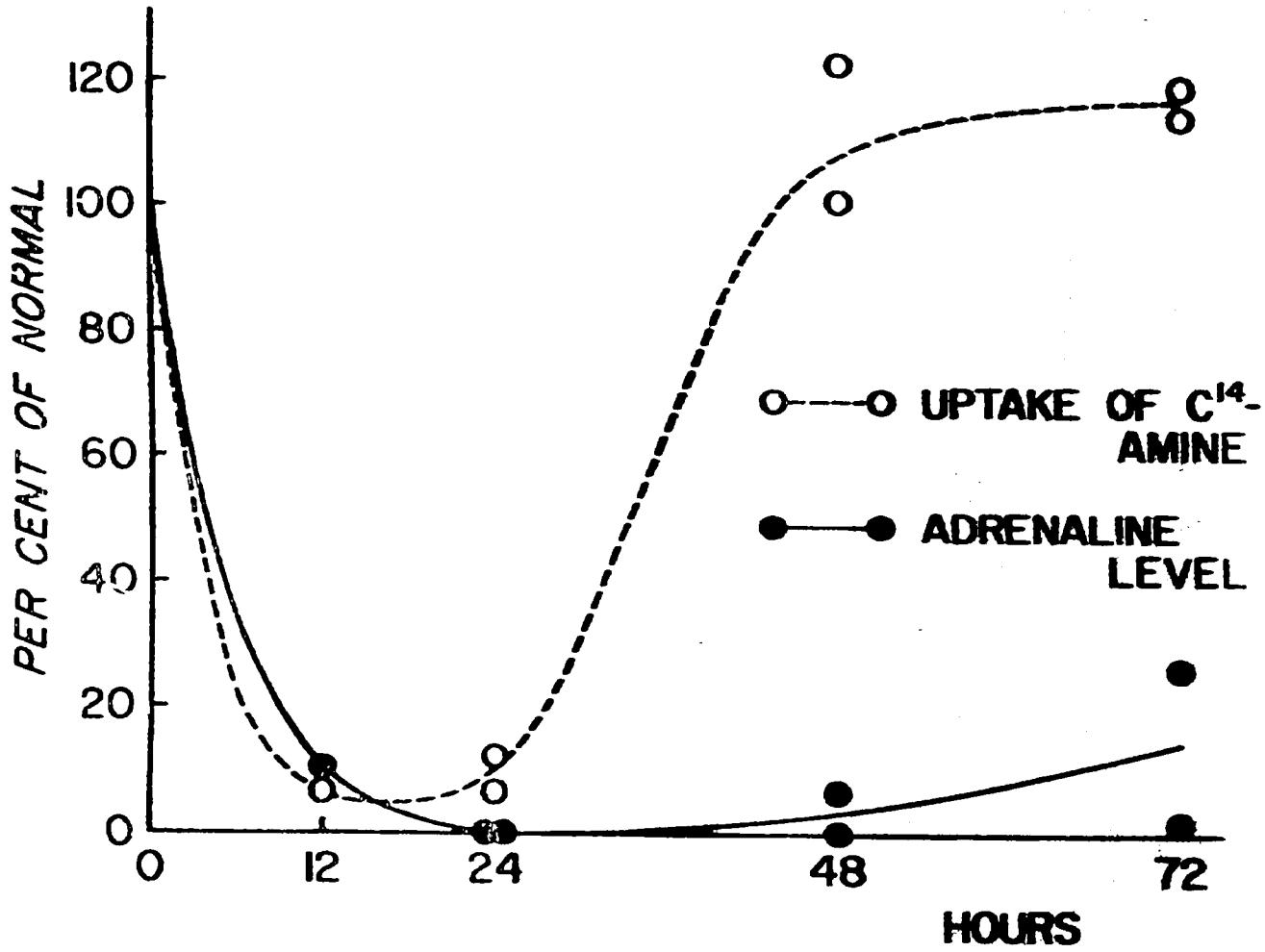


Figure 3. Adrenaline Level of Adrenal Medulla and Uptake of C^{14}-Catecholamines by Adrenal Medullary Granules *In Vitro* at Various Intervals Following Injection of Reserpine (5 mg/kg i.v.) to Rabbits.

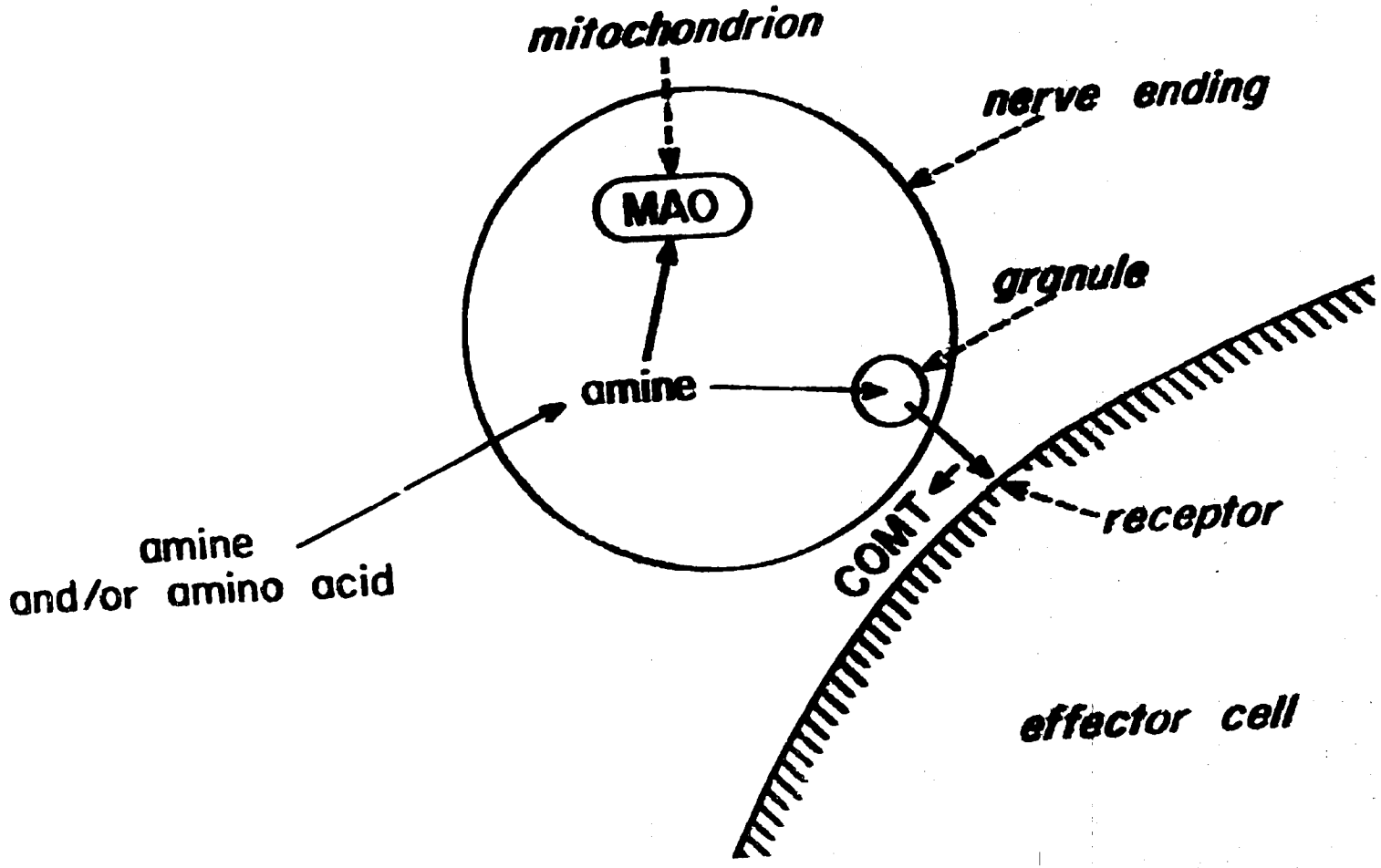


Figure 4. Hypothetical Model of Monoaminergic Transmission Unit.

Attempts to correlate monoamine levels in e.g. the whole hypothalamus, not to mention the whole brain, with functional variables may therefore prove hazardous.

Another common mistake is to identify the noradrenergically innervated centers of the brain with sympathotic centers. This is entirely unjustified as shown by histochemical data. Data on the impulse flow in peripheral sympathetic nerves therefore do not permit any conclusions concerning central noradrenergic mechanisms.

Monoamine precursors.

Even if there are no absolutely reliable tools for investigating the physiological role of brain monoamines, some tools appear to be less hazardous than others. Among the relatively safe tools I should like to mention first the precursors 3,4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylserine (DOPS), and 5-hydrotryptophan (5 HTP). In contrast to the monoamines, their precursors are able to penetrate into brain where they undergo decarboxylation to the respective amines i.e. dopamine (from DOPA), noradrenaline (from DOPS), and 5 HT (from 5 HTP). Injections of these precursors are accompanied by characteristic central and peripheral effects, which are strongly potentiated by monoamine oxidase inhibitors and beyond any doubt mediated by the respective monoamines. Certain objections have been raised against the use of the precursors (Gessa et al., 1962 b). Their decarboxylation appears to be brought about by one and the same enzyme. This would mean, for example, that after injection of 5 HTP, 5 HT would accumulate not only at serotonergic but also at noradrenergic, dopaminergic, and perhaps adrenergic synapses. 5 HT would then be able to activate hypothetical postsynaptic receptors beyond the reach of the 5 HT formed normally. This possibility cannot be excluded at the present time. However, the syndromes produced by 5 HTP and DOPA are markedly different, indicating that different receptors are activated by their respective decarboxylation products. There is no reason to doubt that each amine when formed from the administered precursor, activates its own physiological receptors. It is also reasonable to assume that this activation forms an important feature of the characteristic syndrome of each amine, just as injection of noradrenaline produces a syndrome similar to that caused by stimulation of the noradrenergic system peripherally.

5 HTP causes tremors, convulsions, and hyperextension of the limbs, suggesting that 5 HT neurons participate in the control of motor functions. It is unable to antagonize the akinesia caused by reserpine. DOPA stimulates spontaneous motility and may in suitable dosage restore reserpinized animals almost to normal (Carlsson et al., 1957, Carlsson, 1959). Also more complicated functions such as the conditioned avoidance response are partially restored (unpublished experiments of this laboratory, Seiden). DOPS, which is probably not a physiological precursor of noradrenaline, is decarboxylated very slowly by the decarboxylase. It therefore gives rise to little accumulation of noradrenaline when given alone. After inhibition of the monoamine oxidase it causes accumulation of noradrenaline in both brain and heart. Now both central and peripheral effects are observed, provided that sufficiently large doses are given: for clearcut central actions in mice 1000 mg/kg of the DL-form is needed. The peripheral effects are seen also after smaller doses and correspond to those seen after injection of noradrenaline. They are blocked by phentolamine (in so far as the α-effects are concerned). The central effects are excitatory.

They persist after phentolamine pretreatment. Reserpine-treated animals are awakened and start to move around almost like normal animals. Thus the actions of DOPA and DOPS are similar.

Incidentally, the experiments with DOPS seem to settle the problem whether monoamine oxidase may influence noradrenaline metabolism directly or only via its precursor dopamine. As noradrenaline is formed directly from DOPS, the marked potentiation by a monoamine oxidase inhibitor, indicates that the former alternative is true. This appears to be the case even in peripheral tissues. An influence of catechol-O-methyl transferase on the noradrenaline formed from DOPS is also apparent both in brain and heart, as indicated by increased accumulation of noradrenaline following treatment with an inhibitor of the enzyme (Fig. 5). This will be further discussed later.

The data on monoamine precursors available thus far suggest that dopamine, noradrenaline, and 5 HT are largely excitatory transmitters in the brain. This does not exclude the possibility that also inhibitory actions will be disclosed in the further analysis. The fact that the monoamines counteract rather than mimic the action of reserpine, supports the view that this alkaloid acts by blocking transmission mechanisms of central neurons (just as they do with peripheral neurons) rather than causing an excess of free and active transmitter, as suggested by Dr. Brodie.

Enzyme inhibitors.

Selectively acting enzyme inhibitors should belong to the relatively safe tools for studying monoamine functions in brain. Of course the monoamine oxidase inhibitors have already proved useful. Among these agents nialamide and MO 911 appear to be most selective. They seem to give comparable results. In our laboratory we are mostly using nialamide, which became available first. When these drugs are given to mice in doses sufficient to cause the virtually complete inhibition of monoamine oxidase, the monoamines accumulate rapidly in the brain. The drugs cause central stimulation, as is well known, but again there is no strict temporal correlation with total monoamine levels. Excitation does not seem to set in until the monoamines have already reached high values. This lag may indicate that excess liberation of monoamines to postsynaptic receptors does not set in until the stores have been maximally filled. In support of this the accumulation of normetanephrine has been found to show a similar lag (Carlsson et al., 1960). In any event there is good reason for the assumption that the syndrome caused by a monoamine oxidase inhibitor such as nialamide is mediated by monoamines, since if the accumulation of monoamines is prevented by the administration of agents which inhibit their synthesis (see below), the syndrome does not appear.

Nialamide is able to counteract the sedative effect of reserpine, provided the dose of the latter is not too large. This has been observed in our laboratory in rabbits (Bertler, 1961) and mice (Carlsson, unpublished experiments) and has been confirmed with MO 911 in rabbits by Brodie and Costa, (1962). Both groups of workers agree that the effect shows a better correlation with rise in noradrenaline and/or dopamine than 5 HT. However, 5 HT may very well contribute to the overall effect. In any event the data do not support the view of Brodie and coworkers that the action of reserpine is due to excess free 5 HT, since in animals treated with reserpine followed by a monoamine oxidase inhibitor 5 HT rises much more than the catecholamines. If Dr. Brodie's hypothesis were

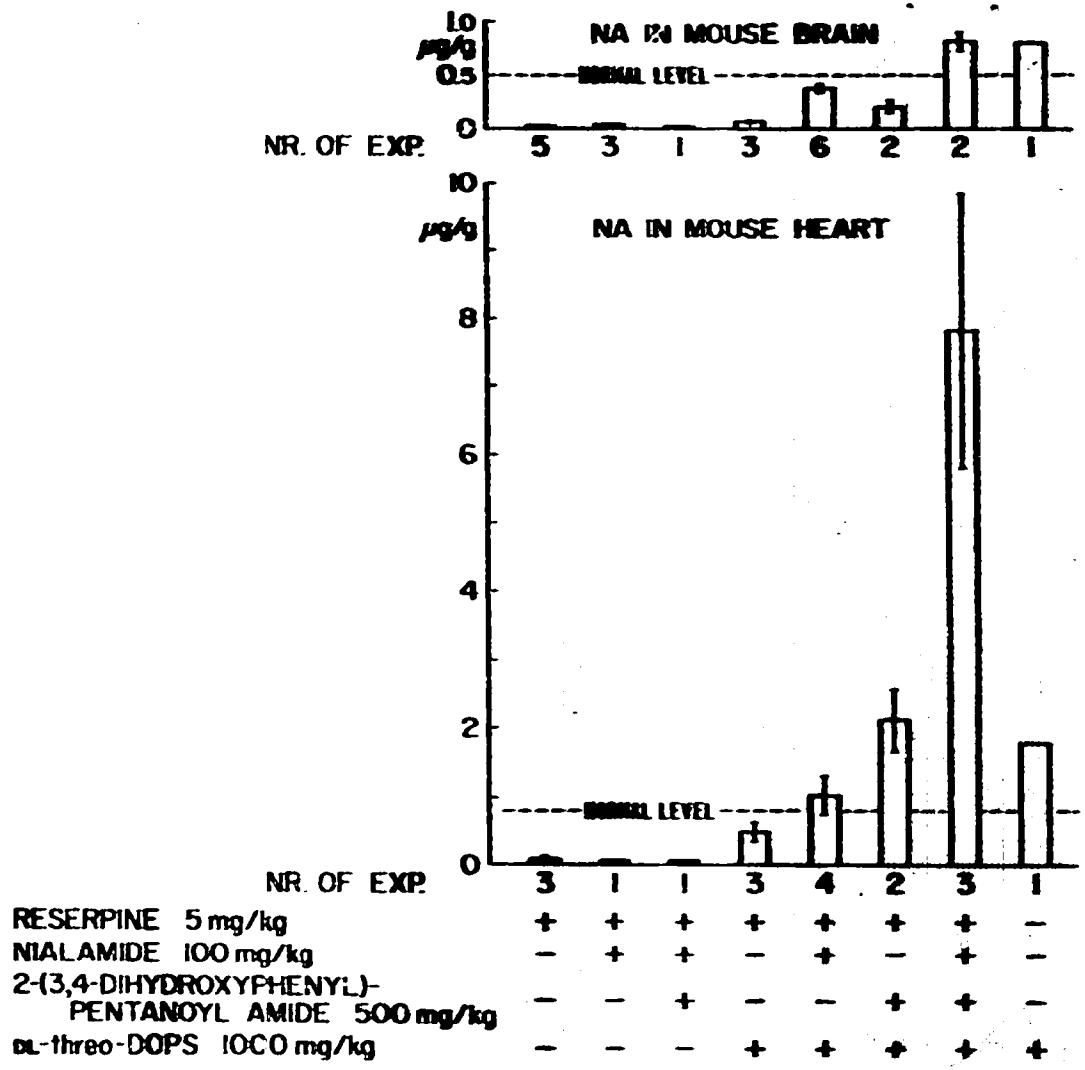


Figure 5. Noradrenaline in Mouse Brain and Heart Following Treatment, Alone and in Various Combinations, with Reserpine (20 hr.), Inhibitors of Monoamine Oxidase (Nialamide, 2 hr.) and Catechol-O-Methyl Transferase (2-(3,4-Dihydroxyphenyl)-pentanoyl Amide, 1.5 hr.), and DL-threo-3,4-Dihydroxyphenylserine (DOPS, 1 hr.) - All drugs were given intraperitoneally. Times above refer to intervals before sacrifice.

correct this should result in aggravation rather than counteraction of the reserpine syndrome.

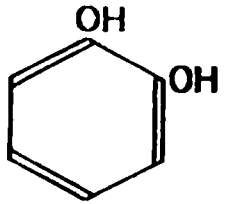
Thus the results with 1) peripheral adrenergic nerves, 2) precursors of the monoamines, and 3) monoamine oxidase inhibitors all point in one and the same direction, namely, that the reserpine syndrome is largely caused by blockade of the transmission mechanism of monoaminergic neurons of different kinds. One further piece of evidence may be added. As is well known, reserpine causes the syndrome of parkinsonism. This may be related to the loss of dopamine, (possibly also 5 HT) from the basal ganglia (Carlsson et al., 1958, Bertler and Rosengren, 1959). In patients suffering from "spontaneous" parkinsonism severe reduction of dopamine and 5 HT has been observed in the basal ganglia (Ehringer and Hornykiewicz, 1960, unpublished data of this laboratory). Treatment of patients suffering from parkinsonism with DOPA results in alleviation of some of the symptoms, particularly the akinesia (Birkmayer and Hornykiewicz, 1961, 1962, Barbeau et al., 1962, unpublished observations of the present research group).

Opinions differ greatly as to the physiological importance of catechol-O-methyl transferase (COMT). While this enzyme seems to be mainly responsible for the breakdown of circulating catecholamines (Axelrod et al., 1958), which appears to be largely due to the high COMT activity of the liver (Axelrod, 1959, Crout et al., 1961, De Schaepdryver and Kirshner, 1961, Carlsson and Waldeck, 1963), it has proved difficult to evaluate its role in other tissues. In fact, Brodie and Costa, (1962) find it unlikely that COMT is even essential for the degradation of circulating catecholamines, owing to the existence of alternative pathways.

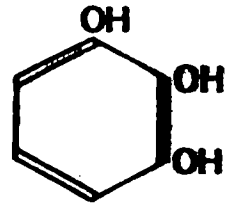
The study of the role of COMT has been greatly hampered by the lack of selective inhibitors of the enzyme. Of the inhibitors available, pyrogallol has been used most extensively. However, data obtained with this agent are often difficult to evaluate, since pyrogallol is toxic and exerts many actions which are unrelated to COMT inhibition. In Göteborg a series of more selective COMT inhibitors has been synthesized by Dr. H. Corrodi (Carlsson et al., 1962a, 1963 a). The compounds are derivatives of 3,4-dihydroxyphenylacetamide (Fig. 6). In non-toxic doses these compounds cause marked inhibition of COMT in vivo (Fig. 7). Several members of the series have a disturbing but interesting "side effect": they inhibit the synthesis of catecholamines and 5 HT in brain. Attempts are being made to separate the two effects.

Normetanephrine and 3-methoxytyramine occur in brain normally and disappear rapidly following COMT inhibition by one of the new compounds (Fig. 8) (Häggendal, 1963 b). The accumulation of dopamine and noradrenaline following administration of DOPA (Fig. 7) and DOPS (Fig. 5), respectively, is increased by COMT inhibition, resulting in potentiation of pharmacological actions of these precursors. It therefore seems likely that COMT is of physiological importance also in brain. In particular, the possibility should be considered that in brain as in the rest of the body COMT is largely responsible for the degradation of extracellular catecholamines, while MAO is responsible for degradation of catecholamines intracellularly near the site of synthesis and storage. Data in support of this hypothesis were put forward a number of years ago (Carlsson et al., 1960, Carlsson, 1960). Later experiments with DOPA (Carlsson and Hillarp, 1962), and now DOPS lend further support to this hypothesis. In this connection it is interesting to note that while reserpine causes an increase in the concentration in brain of acid metabolites formed via the MAO pathway (Roos and

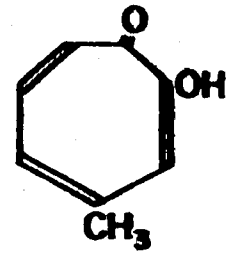
COMT INHIBITORS



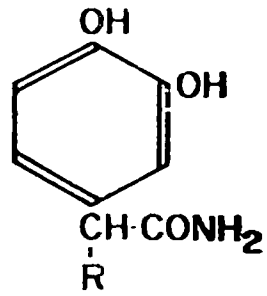
CATECHOL



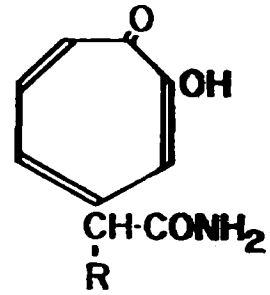
PYROGALLOL



4-METHYLTROPOLONE



DOPACETAMIDE SERIES



TROPOLONEACETAMIDE SERIES

Figure 6. Catechol-O-Methyl Transferase Inhibitors of Current Interest. Catechol and pyrogallol have been most widely used so far. 4-methyltropolone is a potent inhibitor described by Belleau and Burba (1961). The dopacetamide and tropoloneacetamide series have been synthesized by Dr. H. Corrodi, Ekssle Ltd., Göteborg, Sweden.

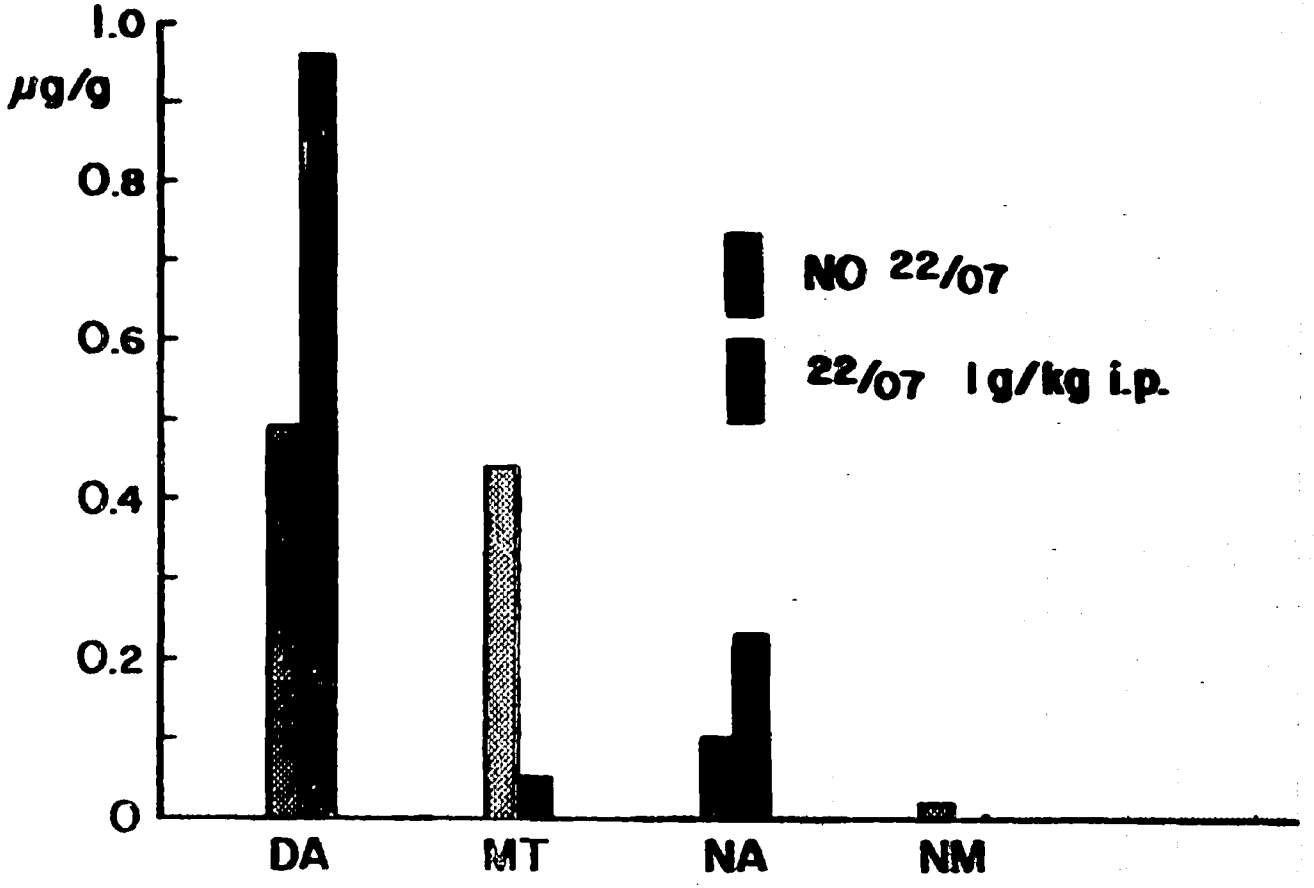


Figure 7. Effect of L-DOPA (7.5 mg/kg; 1 hr.) on Dopamine (Da), 3-Methoxytyramine (MT), Noradrenaline (NA), and Normetanephrine (NM) Levels in House Brain Following Pretreatment with Reserpine (25 mg/kg; 20 hr.), Nialamide (100 mg/kg; 2 hr.), and α-ethoxydopacetamide (22/07; 1 g/kg, 1.5 hr.). – All drugs were given intraperitoneally. Times above refer to intervals before sacrifice.

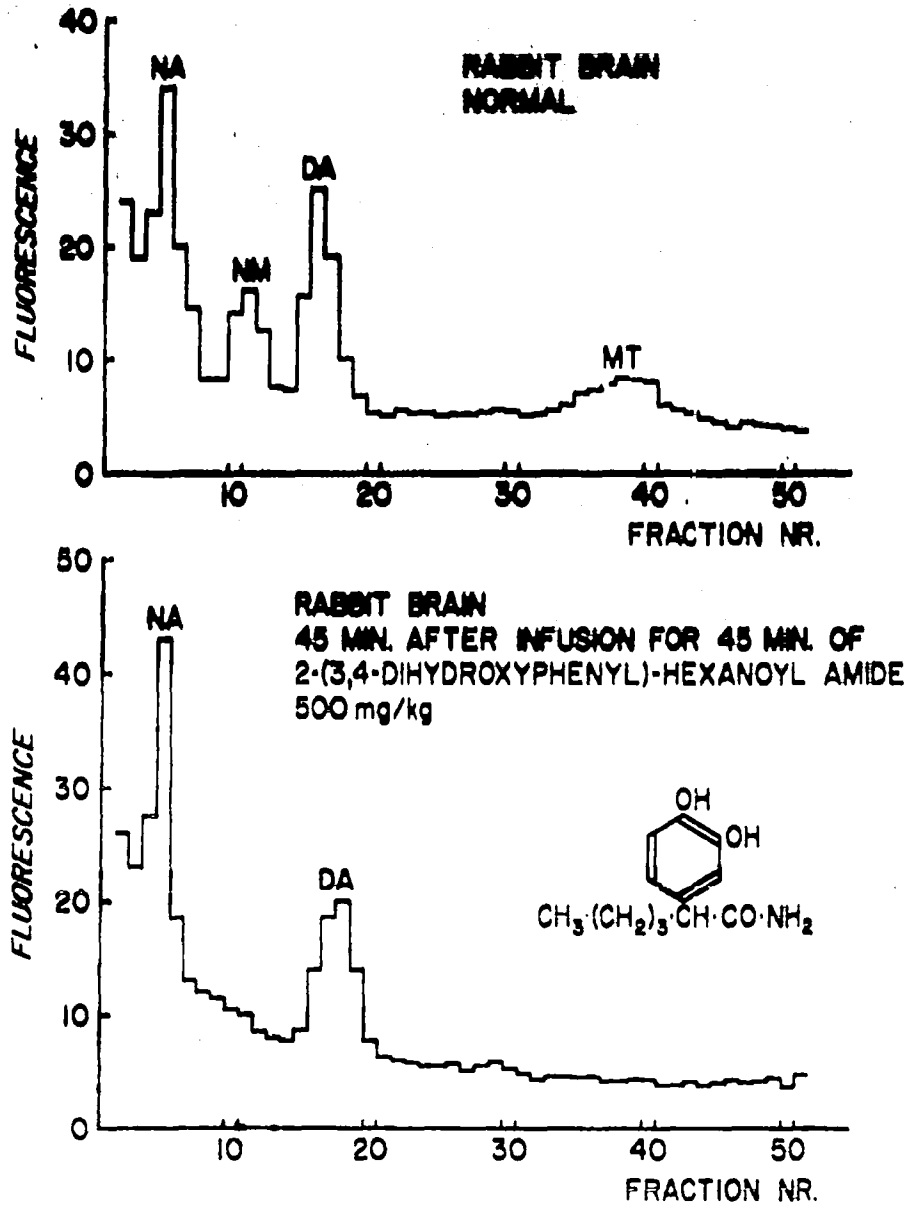


Figure 8. Noradrenaline (NA), Normetanephrine (NM), Dopamine (DA), and 3-Methoxytyramine (MT) in Rabbit Brain Normally and After Treatment with an Inhibitor of Catechol-O-Methyl Transferase (Measured, 1963 b).

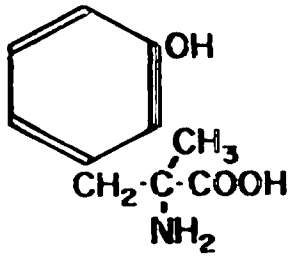
Nordinius, 1962, Ashcroft and Sharman, 1962), it has been found to cause a decrease in the concentration of 3-O-methylated metabolites of catecholamines (Häggendal, 1963 b). This seems to indicate that reserpine causes a decrease in the liberation of catecholamines into the extracellular space, and thus to receptor sites, and at the same time a net increase in the release from the granules to the cytoplasmic sap, from where they can penetrate into the mitochondria and form a substrate for the MAO.

Needless to say, it would be of great theoretical and perhaps also practical interest to have efficient inhibitors of enzymes responsible for the synthesis of monoamines in brain. A number of years ago it was generally thought that the fall in monoamine levels caused by α-methyl DOPA and α-MMT was brought about by inhibition of DOPA decarboxylase. Later it was found, however, that this effect could be at least partly accounted for by the release or displacement mechanism discussed earlier. Today the view seems to be favored that inhibition of synthesis is of no great importance for the action of these DOPA analogs. One argument supporting this view is that we know of a series of DOPA decarboxylase inhibitors (the "NSD compounds", Fig. 9) which are unable to produce a fall in monoamine levels, although they are more potent inhibitors of the enzyme (Drain et al., 1962, Brodie et al., 1962 a). Although the decarboxylation of exogenous precursor is largely blocked by these compounds, it appears that normal synthesis is unimpaired, suggesting that the enzyme is present in large excess of the normal needs.

We have reinvestigated the problem using a biochemical in-vivo method, i.e., as an indicator of decarboxylase activity we have used the accumulation of 5 HT following 5 HTP administration (50 mg/kg i.p.) to mice pretreated with a MAO inhibitor (nialamide 100 mg/kg i.p. 30 minutes before the 5 HTP). The drug to be tested for decarboxylase activity was given i.p. 30 minutes before the MAO inhibitor. Both brain and kidney were examined. The results are expressed as per cent inhibition (Fig. 10). Percentage inhibition was set to zero when the accumulation of 5 HT was the same as in animals given the MAO inhibitor and 5 HTP only. It was set to 100 per cent if 5 HT values were the same as in untreated normal animals.

Of the 3 DOPA analogues tested, α-methyl DOPA was clearly more efficient than α-MMT and MK 485 (the hydrazine analogue of α-methyl DOPA, Porter et al. 1962), particularly in brain. Of the NSD compounds, NSD 1015 (m-hydroxybenzylhydrazine) was clearly more efficient than NSD 1034, i.e. its N-methyl derivative, and NSD 1024 (m-hydroxybenzyloxyamine). No data on NSD 1015 seem to have been published before. According to the present data this compound is a more potent inhibitor of the decarboxylase than α-methyl DOPA. Unlike α-methyl DOPA, however, it was unable to produce a decrease in brain 5 HT. It also proved to be unable to block the fall in brain 5 HT caused by α-methyl DOPA, although it blocked the prolonged and pronounced fall in noradrenaline, apparently by blocking the decarboxylation of α-methyl DOPA. This suggests that the fall in 5 HT is caused by a direct action of α-methyl DOPA rather than by its decarboxylation products. In our laboratory Roos and Nordinius, (1963), have found that treatment of rabbits with α-methyl DOPA results in a drop in both 5 HT and 5-hydroxyindoleacetic acid. Similar observations have been made by Sharman and Smith (1962). This argues against release or displacement and favors the view that α-methyl DOPA inhibits the synthesis of 5 HT. If this inhibition cannot be accounted for by decarboxylase inhibition, as the experiments with NSD compounds suggest, then we seriously have to consider the possibility that α-methyl DOPA inhibits the first step in the

DECARBOXYLASE INHIBITORS



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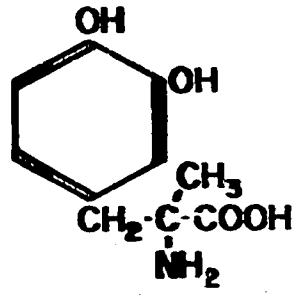
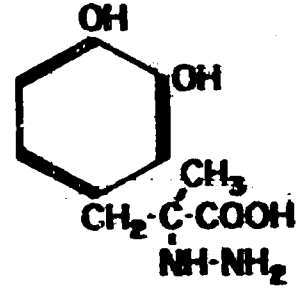


α-METHYLDOPA



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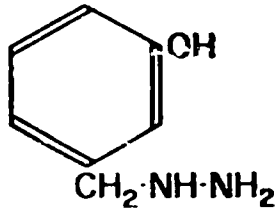

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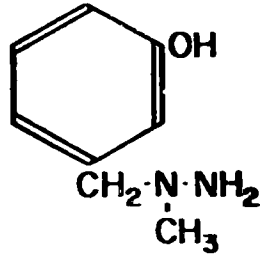
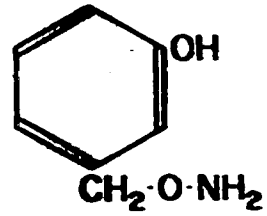


NSD 1034



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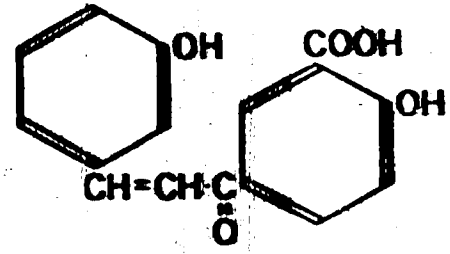

HCS

Figure 9. DOPA Decarboxylase Inhibitors of Current Interest. - HCS = 3-hydroxycinnamoyl salicylic acid (Clark, 1959).

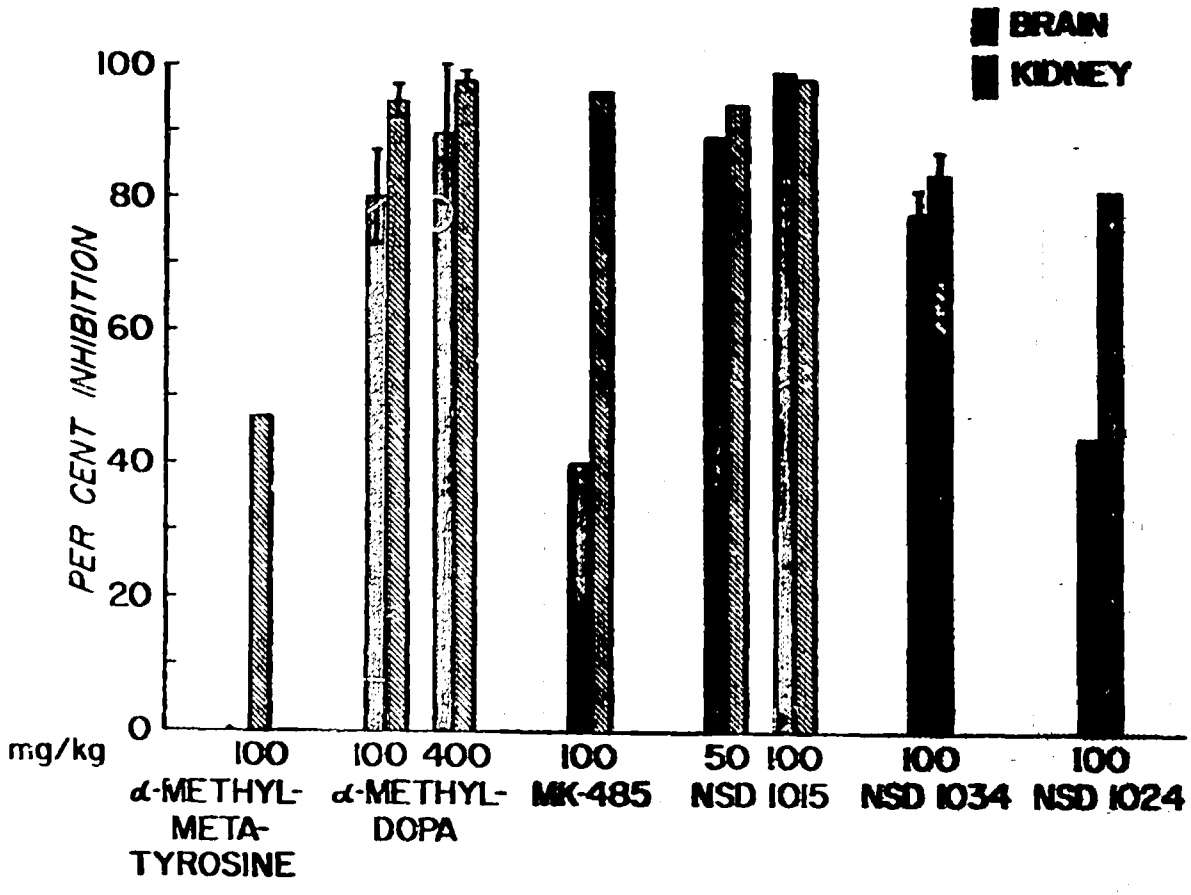

Figure 10. In-Vivo Activity of Decarboxylase Inhibitors.

synthesis of 5 HT, i.e., the hydroxylation of tryptophan on the 5 position. In any event, the data so far available indicate that at least two mechanisms are at work in the fall in monoamine levels brought about by α-methyl DOPA and α-MMT, namely, a) inhibition of synthesis caused by a direct action of the amine acids, and b) displacement caused by their decarboxylation products. Which of these effects, if any, is responsible for the fall in blood pressure and sedation caused by α-methyl DOPA is not known, although inhibition of synthesis appears to be the more likely alternative.

As already mentioned, a number of dihydroxyphenylacetamide derivatives have been found to inhibit not only COMT but also the synthesis of monoamines in brain. There is no inhibition of the decarboxylase, so the site of attack is probably the first step in the synthesis, i.e. the hydroxylation of trypotphan on the 5 position and the hydroxylation of tyrosine on the 3 position, respectively. The evidence for inhibition of synthesis is 1) fall in 5 HT, dopamine, and noradrenaline levels in brain, 2) block of the accumulation of monoamines in brain caused by inhibition of MAO (Carlsson et al., 1962 c, 1963 a, Fig. 11), and 3) fall in 5-hydroxyindoleacetic acid level in brain (Roos and Werdinius, 1963). The compounds have a depressant effect on the central nervous system, but it is not known if this effect is caused by the inhibition of monoamine synthesis.

Experiments with brain lesions.

The localization of noradrenaline, and probably dopamine and 5 HT, to neurons in the brain has prompted us to investigate the noradrenaline (Magnusson and Rosengren, 1963) and 5 HT (Carlsson et al., 1963 b) levels of the spinal cord of rabbits following transection at the level of the second thoracic segment. Both noradrenaline and 5 HT were found to disappear almost entirely below the lesion but were unchanged above the lesion, indicating the existence of descending noradrenergic and serotonergic pathways in the spinal cord (Fig. 12). Intravenous injection of L-DOPA (100 mg/kg) was followed by marked stimulation of spinal reflexes below the lesion, suggesting a facilitating function of noradrenergic neurons. Likewise, injection of 5 HTP caused stimulation of spinal reflexes, although the picture appeared to be qualitatively different from that caused by DOPA.

Recently Heller et al., (1962), reported that destruction of the medical forebrain bundle within the lateral hypothalamus of the rat produced a fall of 36 per cent in brain 5 HT levels as compared with normal controls.

Concluding speculations.

The present data suggest that the monoamine-storing granules have a dual function, namely a) to serve as a store of monoamines, and b) to facilitate the transfer of monoamines from the site of synthesis to the site of liberation into the synaptic cleft. The significance of the first function is dubious, as the organism apparently can do well without the store. It may possibly be of importance under special emergency conditions, e.g. if the synthesis of transmitter or its transfer from the site of synthesis to the site of liberation into the synaptic cleft is blocked. This may possibly be the case during the early stage of reserpine action. It will also be interesting to see, if displacement of the noradrenergic transmitter by a less active analogue is accompanied by increased sensitivity to agents which inhibit the synthesis of transmitter. Preliminary observations suggest that this may be so.

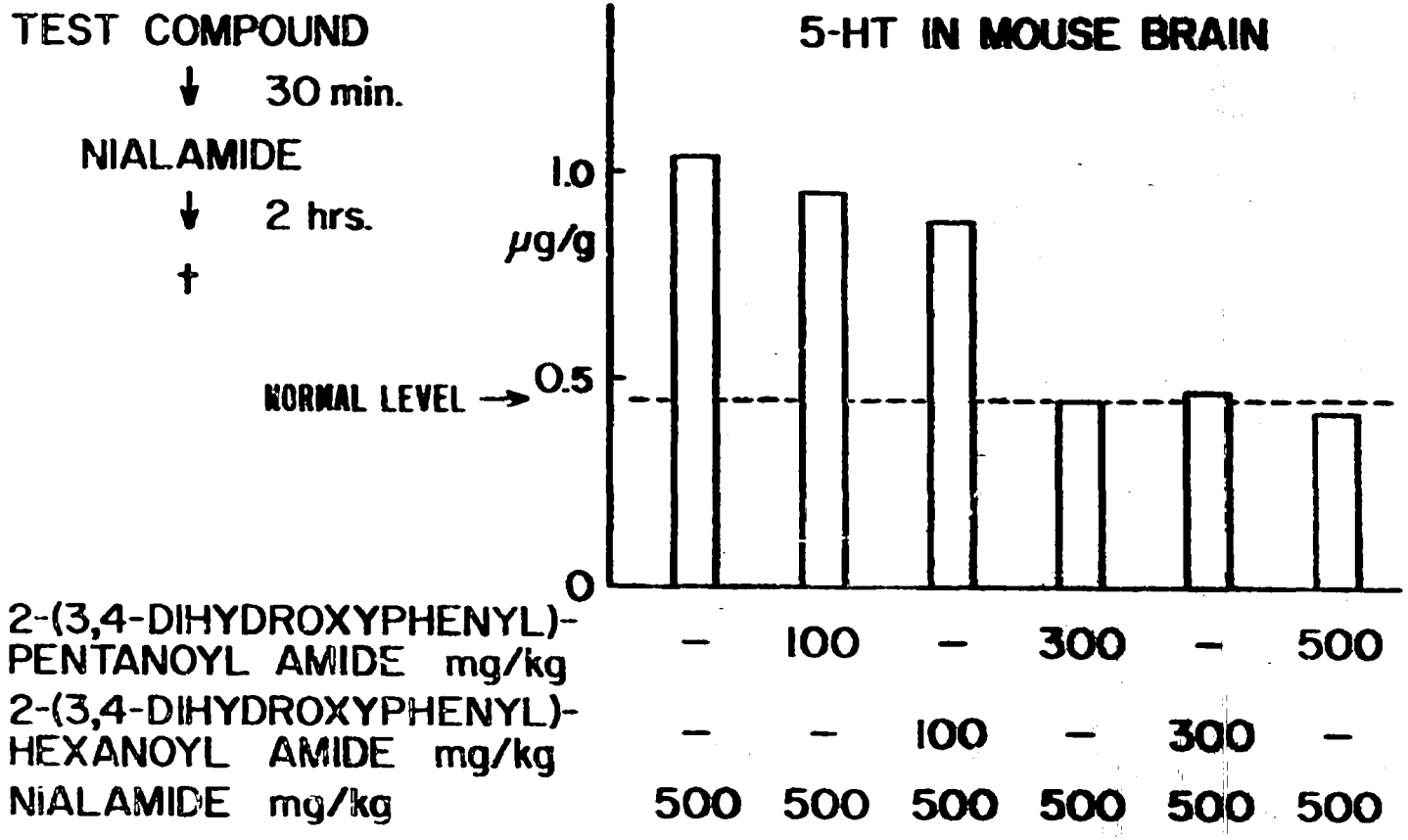

Figure 11. Blockade by Two Inhibitors of Catechol-O-Methyl Transferase of the 5-Hydroxytryptamine Accumulation Induced in Brain by Inhibition of Monoamine Oxidase.

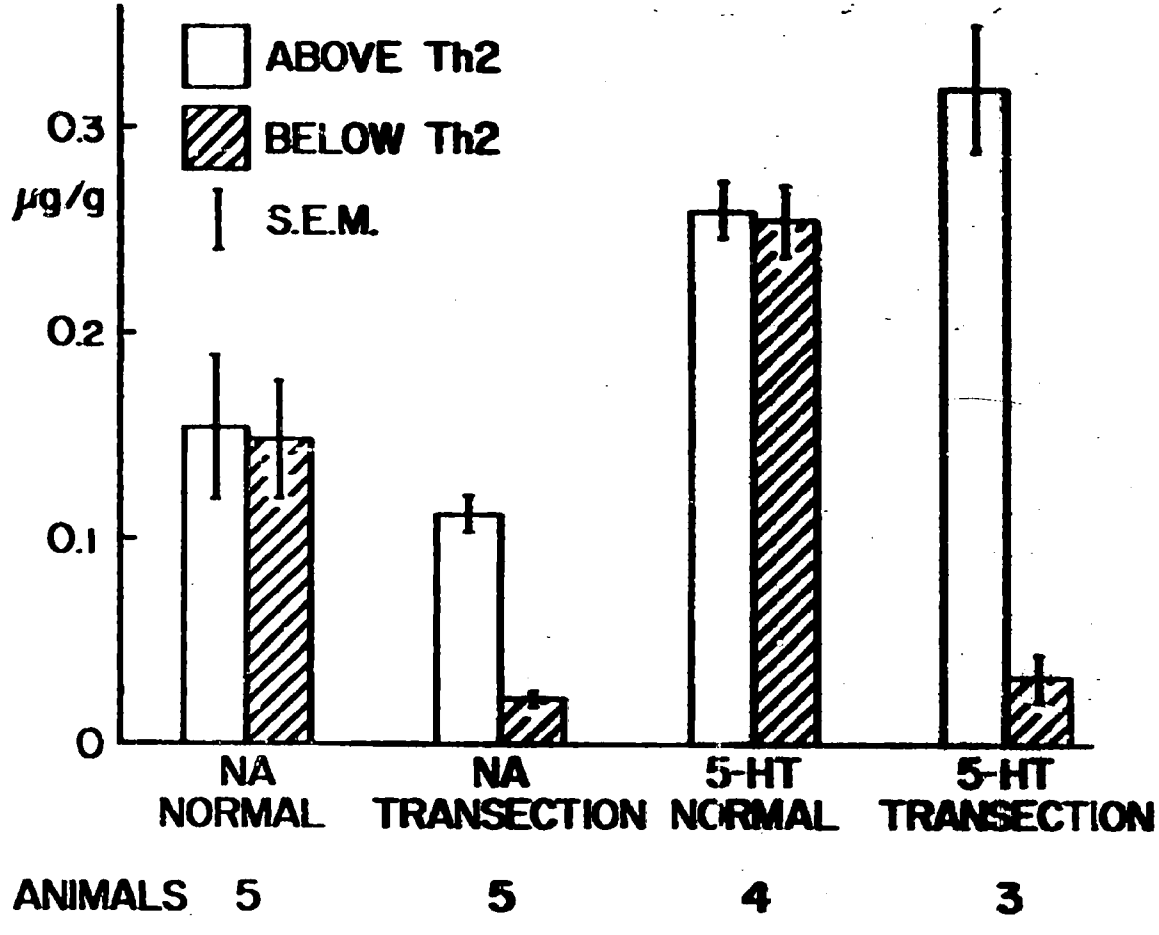

Figure 12. Noradrenaline and 5-Hydroxytryptamine in Rabbit Spinal Cord Normally and After Transection at Second Thoracic Segment. (Magnusson and Rosengren, 1963, Carlsson et al. 1963 b).

In rats the injection of α-methyl DOPA (400 mg/kg i.p.) causes but slight sedation and ptosis. A second injection of the same dose 24 hours later causes clearcut sedation and ptosis (Andén and Magnusson, unpublished experiments). This may be interpreted to mean that the first injection causes inhibition of synthesis, but transmitter functions are unimpaired since they may proceed at the expense of the stores. At the time of the second injection, however, the noradrenergic transmitter has been replaced by the less active α-methyl analogue in the store. When synthesis is blocked by the second injection, the transmission mechanism loses much of its efficiency.

The possibility should be considered that the store of transmitter cannot always be mobilized at sufficient rate to keep transmission intact, should the synthesis or the uptake by the granules be blocked e.g. by a drug. It is remarkable that symptoms of depression may set in before the monoamine stores are emptied. To a certain extent this is true of reserpine, but even more so of the benzoquinolizines. Actually some of the benzoquinolizines, for example, benzoquinamide, have been stated to depress brain functions in doses which do not cause any decrease at all in brain monoamine levels (Weissman and Finger, 1962, Plotscher et al., 1962).

In connection with the benzoquinolizines it is interesting to note that the dihydroxyphenylacetamide derivatives mentioned before cause depression of the central nervous system and inhibition of monoamine synthesis. The symptoms of depression seem to reach their maximum before the monoamine levels have reached their minimum, which is in analogy with the benzoquinolizines. Of course it is possible that no causal relationship exists between the biochemical and behavioral effects in the case of these two groups of drugs. The possibility cannot be excluded, however, that a small number of highly active and functionally essential neurons are almost exclusively dependent on newly synthesized transmitter, possibly because they are unable to mobilize the store at sufficient speed. It may thus be as difficult to disprove as to prove a causal relationship between biochemical and behavioral effects of drugs.

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Terms and abbreviations.

dopamine	β(3,4-dihydroxyphenyl)ethylamine
noradrenaline	β(3,4-dihydroxyphenyl)β-hydroxy ethylamine
adrenaline	β(3,4-dihydroxyphenyl)β-hydroxy N-methyl ethylamine.
metanephrine	β(3-methoxy-4-hydroxyphenyl)β-hydroxy N-methyl ethylamine
normetanephrine	β(3-methoxy-4-hydroxyphenyl)β-hydroxy ethylamine
DOPA	β(3,4-dihydroxyphenyl)alanine
DOPS	β(3,4-dihydroxyphenyl)serine
α-MMT	α-methyl metatyrosine
metaraminol	α-methyl β-hydroxy metatyramine
MK 485	α-methyl α-hydrazino β(3,4-dihydroxyphenyl) propionic acid
DOPAC	3,4-dihydroxyphenylacetic acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
5-HIAA	5-hydroxyindoleacetic acid
MAO	monoamine oxidase
COMT	catechol-O-methyl transferase
NSD 1015	m-hydroxybenzyl hydrazine
NSD 1024	m-hydroxybenzyl oxyamine
NSD 1034	m-hydroxybenzyl N-methyl hydrazine

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Arvid Carlsson April 10, 1963

ABSTRACT: The mechanism of monoaminergic (i.e. adrenergic, noradrenergic, dopaminergic, and serotonergic) transmission in general is discussed on the basis of observations on the effects of various agents (chiefly precursors and precursor analogs,

inhibitors of enzymes responsible for synthesis and degradation of monoamines, and drugs interfering with storage and release). The storage granules (or vesicles) of the monoaminergic nerve terminals, in which the transmitter can be visualized under the fluorescence microscope by means of a new histochemical technique, appear to have a dual function, i.e. a) to serve as a store of transmitter, and b) to make newly synthesized transmitter available for release by membrane depolarization. The former function does not seem to be essential, since the store can be depleted without any disturbance of transmission. The latter function seems to be essential: block of the uptake mechanism of the granules by reserpine results in block of transmission.

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